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Evaluation of Phytochemical, Antidiarrheal and Antimicrobial Properties of *Tinospora cordifolia* in Albino Rats

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ABSTRACT

Tinospora cordifolia (Family Menispermaceae) commonly known as Amrita (Guduchi, Giloya) is widely used by tribals for the treatment of various infectious diseases. The plant also possess various pharmacological activities including its use as antihyperglycemic, anti-inflammatory, antiarthritic, antimicrobial, antiosteoporotic, enhance cognition (learning and memory), antidiarrhoeal and immunomodulatory effects. Our aim was to investigate the antidiarrheal, and antimicrobial activities of methanolic extract of *Tinospora cordifolia* (GURJO) in albino rats. The present study was carried out to study the phytoconstituents properties of *Tinospora cordifoila* leaf. *Tinospora cordifolia* leaf collected from nandidurg (nandi hills) in the south Indian state of Karnataka. Phytoconstituents screening revealed the presence of alkaloids, saponins, glycosides, carbohydrate, proteins and amino acids, phytosterol, phenol, flavonoids and diterpenes. The antidiarrheal effect was evaluated by using castor oil-induced diarrhoea, and gastrointestinal motility tests at 200 mg/kg and 400 mg/kg body weight in rats. The leaf extract showed considerable antidiarrheal effect by inhibiting 40% and 60% of diarrheal episode at the doses of 200 and 400 mg/kg, respectively. *Tinospora cordifolia* also significantly reduced the castor oil-induced intestinal volume in enteropooling test as well as intestinal transit in GI motility test, compared to their respective control. These observed effects are comparable to that of standard drug loperamide (3mg/kg). So these results indicate that bioactive compounds are present in methanolic extract of *Tinospora cordifolia* including significant antidiarrheal activity, and antibacterial activity could be accounted for pharmacological effects. The results will be calculated using statistical analysis by applying Annova and student t-test. P value for all was found to be <0.01. Thus it was observed that extract was having a highly significant antidiarrheal and antimicrobial effect as evident from the p-value. Traditionally people used *Tinospora cordifolia* for the treatment of various ailments, including diarrhoea.

INTRODUCTION:

Herbal plants produce and contain a variety of chemical substances that act upon the body. Herbalists use the leaves, flowers, stems, berries, and roots of plants to prevent, relieve, and treat illness. Diarrhoea is the passage of three or more loose stools. [1, 2] It is characterized by increased gastrointestinal motility and secretion and a decrease in the absorption of fluid and electrolytes. [3, 4] It is usually a symptom of gastrointestinal infection, which can be caused by a variety of bacterial, viral and parasitic organisms. Infection is spread through contaminated food or drinking-water, or from person to person as a result of poor hygiene. Severe diarrhoea leads to fluid loss, and maybe life-threatening, particularly in young children and people who are malnourished or have impaired immunity. Conservative estimates place the global death toll from diarrhoeal diseases at about two million deaths per year (1.7 - 2.5 million deaths), ranking third among all causes of infectious disease deaths worldwide. Most of these deaths occur in children under five years of age. An average morbidity attack rate of 3.2 episodes of diarrhoea per year per child has been reported. Medicinal plants are a promising source of new antidiarrheal drugs. WHO studies the treatment and prevention of diarrheal diseases using traditional medical practices. Currently available drugs are linked with adverse effects and contraindications. Drug resistance is another challenge to think about antibiotics used in the treatment of diarrhoea. [5] The high incidence of diarrhoea in developing countries coupled with limitations of currently available antidiarrheal drugs and poor healthcare coverage may make traditional medicines good alternative agents for the management of diarrhoea.

TYPES OF DIRRHOEA

There are six types of diarrhoea:

Secretory Diarrhoea: Secretory diarrhoea means that there is an increase in the active secretion, or there is an inhibition of absorption. The most common cause of this type of diarrhoea is a cholera toxin that stimulates the secretion of anions, especially chloride ions.

Osmotic Diarrhoea: Osmotic diarrhoea occurs when too much water is drawn into the bowels. If a person drinks solutions with excessive sugar or excessive salt, these can draw water from the body into the bowel and cause osmotic diarrhoea. [6]

Exudative Diarrhoea: Exudative diarrhoea occurs with the presence of blood and pus in the stool. This occurs with inflammatory bowel diseases, such as Crohn's disease or ulcerative colitis, and other severe infections such as *E. coli* or other forms of food poisoning.

Motility-related Diarrhoea: Motility-related diarrhoea is caused by the rapid movement of food through the intestines (hypermotility).

Inflammatory Diarrhoea: Inflammatory diarrhoea occurs when there is damage to the mucosal lining or brush border, which leads to a passive loss of protein-rich fluids, and a decreased ability to absorb these lost fluids.

Dysentery Diarrhoea: Generally, if there is blood visible in the stools, it is not diarrhoea, but dysentery. [7] The blood is a trace of an invasion of bowel tissue. Dysentery caused by various microbes especially organisms belonging to *Shigella* spp., *Entamoeba histolytica*, and *Salmonella* species. [8, 9]

SYMPTOMS OF DIARRHOEA

The common symptoms of diarrhoea include the following.

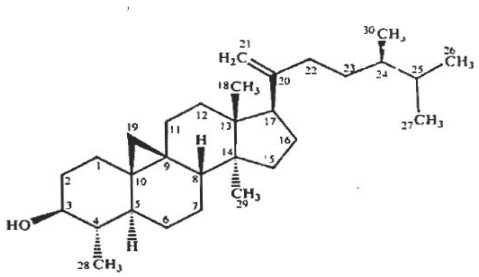
Abdominal cramps
abdominal pain
An urge to go to the toilet, sometimes this may be sudden
Loss of appetite
Fatigue
Loose, watery stools
Bloating
Blood in stool

Tinospora cordifolia, which is commonly known as Giloy or Gudachia. [10] It belongs to the family of Menispermaceae and is a vigorous climber. Its English name is *Gulancha Tinospora*. There are about 40 species of Giloy that are found throughout the world, comprising parts of Africa, Southern Eastern Asia, and Australia. Out of 40 species only 4 species have been found in India. These consist of: (i) *Tinospora cardifolia*; (ii) *T. sinensis*; (iii) *T. malabarica*; and (iv) *T. tomentosa.*

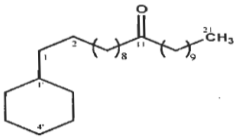
Figure No. 1: *Tinospora cordifolia*

As *Tinospora cordifolia* traditional medicine to be used in the control of diarrhoea and dysentery. Hence in our study, we are screening of leaf of *Tinospora cordifolia* for its antidiarrheal and antimicrobial activity. [11] This is in continuation with the exploration of a natural resource to combat drug resistance. [12, 13]

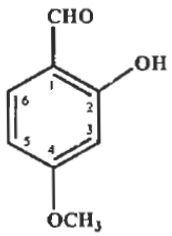
Structure of chemical constituents of *Tinospora cordifolia*:



cycloeuphordenol



Cyclohexyl-11-heneicosanone



2-Hydroxy-4-methoxy-benzaldehyde

Preparation of extract: Soxhlet extraction is only required where the desired compound has limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. The powdered leaf material of *Tinospora cordifolia* was placed in a thimble and extracted with 70% methanol in a Soxhlet apparatus for 70-72 hrs. Solvents were removed in water bath. The residue (extract) of the respective plant material was stored at 4°C until used. The extract yield (% w/w) from the plant material was recorded as 13.2%.

Figure No. 2: A picture of Soxhlet apparatus during an extraction

Preliminary Phytochemical Screening: The preliminary phytochemical screening was carried out using the powered leaf for different types of chemical constituents. The qualitative chemical test gives the general idea regarding the nature of chemical constituents of the crude drug. The extracts were subjected to preliminary phytochemical investigation for detection of Alkaloids, Carbohydrates, Glycosides, Phenolic compounds, Flavonoids, Proteins and amino acids, Saponins, Steroids. The phytochemical tests were carried out in four solvents such as Water, Methanol, Benzene, and Petroleum ether. The extraction of powdered was decided after phytochemical test to determine the solvent for mass extraction.

Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood-red colour indicates the presence of cardiac glycosides.

Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Detection of phytosterols

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. The appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish-black colour indicates the presence of phenols.

Detection of tannins

Gelatine Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of proteins and amino acids

Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

PHARMACOLOGICAL ACTIVITY

Antidiarrheal activity

Experimental animals: Wistar albino rats of either sex weighing between 125-250 gm were used for the study. They were housed in propylene cages at 25 ± 2 ºC with 12 hrs light and 12 hrs dark cycle. All the animals were fed with standard feed and water *ad libitum*. All the animals were maintained under standard laboratory condition.

Figure No. 3: A picture of Wistar albino rats which is kept in the cages

Drugs and Doses

Standard drug: Loperamide (3mg/kg), drug is dissolved in 10 ml distilled water and the drug is given to the rats according to the body weight.

Normal saline solution: 0.9% w/v, the NaCl is dissolved in 100ml of distilled water and the saline is given to the rats according to body weight.

Test drug: The extract was made suspension in the distilled water. Two doses of the extract were selected 250mg/kg bw. and 500mg/kg bw.

Castor oil: 1ml of castor oil given to each rat to induce diarrhoea.

All the drug, extract, normal saline solution and castor oil were given to the rats by oral route.

MATERIALS AND METHODS:

Animals: The albino rats (either sex) 200-250g

Chemicals: Loperamide, Normal saline, DMSO, Ciprofloxacin, Methanol and Distilled water.

Apparatus: Hot air oven, B.O.D. incubator, Laminar air flow, Soxhlet apparatus.

Castor oil-induced diarrhoea model: Albino rats of either sex (200-250g) were divided into four groups of six animals each. They were fasted for 24h prior to the test, but allowed free access to water.

Group 1 Received control: Normal saline (0.9%w/v)

Group 2 Received standard drug (Loperamide 3mg/kg).

Group 3 Received dose of the extract (200mg/kg)

Group 4 Received dose of the extract (400mg/kg)

All doses were administered orally. The animals were then housed singly in cages lined with transparent paper. One hour after pre-treatment with the extract, the animals were challenged with 1 ml of castor oil orally. Stools were collected on nonwetting paper sheets of uniform weight up to 24 h after administration of the castor oil. Every 15min during the first 8 h, urine was drained off by gravity, and the net stool weight, termed early diarrhoeal excretion, was recorded. The diarrhea-free period is defined as the time in minutes between castor oil administration and the occurrence of the first diarrhoeal output. The acute diarrhoeal phase is the time between the first and the last diarrhoeal output of the 8-h observation period. Stools occurring between 8 and 24 h after castor oil administration are called late diarrhoeal excretion.

***IN-VITRO* ANTIMICROBIAL ACTIVITY:** The antimicrobial activity of the methanolic extract of *Tinospora cordifolia* was determined by the minimum inhibitory concentration of the extract against 25 bacterial strains.

Test micro-organism

List of test- organism for *in-vitro* antimicrobial activity:

S. No	Name of micro-organism
1.	*Pseudomonas auriginosa* AP585 NLF
2	*Pseudomonas putiba* MTCC 2252
3	*Vibrio cholera* 575
4	*Vibrio cholera* 1033
5	*Vibrio cholera* 426
6	*Vibrio cholera* 765
7	*Vibrio cholera* 1023
8	*Vibrio cholera* 1311
9	*Vibrio cholera* BDI/81
10	*Shigella soneii* NK29
11	*Shigella soneii* BCH 397
12	*Shigella soneii* F11001
13	*Shigella dysenteriae* 1
14	*Shigella boydii* 22461
15	*Shigella flexneri* type 36 NK 381
16	*Bacillus cereus* MTCC 1305
17	*Bacillus subtilis* MTCC 441
18	*Escherichia coli* 306
19	*Escherichia coli* 798
20	*Escherichia coli* 35B
21	*Escherichia coli* 18/9
22	*Actinobactor spp*
23	*Protious vulgaris* AP769 NLF
24	*Klebsiella pneumoninae*
25	*Salmonella typhii* type II

Preservation of bacterial culture:

All the strains were preserved as stab slant culture at a temperature of 4 ºC. All these strains were checked for purity and identified by gram staining and standard biochemical tests. Routine subculturing of gram-positive bacteria was carried out on nutrient agar and of gram-negative strain on bromothymol blue lactose agar.

The agar dilution technique for assessment of antibacterial activity:

The minimum inhibitory concentration of various extracts against the bacterial strains was determined by the checker board technique as described below.

Preparation of stock solution of extracts:

Desired amount of each of the methanolic extracts of leaf was dissolved in dimethyl sulphoxide (DMSO) to prepare the stock solution of 1 mg/ml, 10 mg/ml and 20 mg/ml. Suitable dilution made from this to get various dilution of 5, 25, 50, 100, 200 and 400 µg/mL.

Preparation of ciprofloxacin solution as standard:

Standard ciprofloxacin taken and various dilutions of 400µg/mL and 800µg/mL in DMSO were during disk diffusion study.

Preparation of nutrient agar plate containing different concentration of the extract required for determination of minimum inhibitory concentration (MIC) of the extracts with respect to different bacteria:

Measured volumes of stock solution of the extract were individually added aseptically to molten nutrient agar in the following concentration (µg/mL): 0 (control), 5, 25, 50, 100, 200 and 400 µg/mL poured into sterile petri dishes. The pH of the media was adjusted to 7.2-7.4. For uniform diffusion of the extract throughout the medium, the agar plates containing extracts were refrigerated overnight and subsequently dried for 2 hours at 37 ºC in B.O.D. incubator before use. Small squares were demarcated at the back of agar containing a portion of the plates with a marker to specify the actual location for each test organism.

Figure No. 4: Hot air oven

Figure No. 5: B.O.D. incubator

Inoculum:

The inoculum for determination of the sensitivity pattern consisted of one loopful of an overnight grown broth culture of the test organism. The average size of the inoculum was about 10^5 cells contained in a 2 mm diameter loop.

Spot inoculation method:

The nutrient agar plate containing the extract and the solvent (control plates) having equal volume of were made ready and kept overnight to detect the presence of contamination. The overnight grown broth culture of each test organism was spot inoculated by checkerboard

technique on the marked area of the 100 mm petri plates. These were then incubated for 72 hours at 37 °C. No growth of the organism on the test plate along with growth on the control plates was taken as an indication of antimicrobial activity of the extract. The readings were recorded in a tabular form. Minimum inhibitory concentration (MIC) was indicated by the lowest concentration of the extract with inhibited the bacterial growth.

Figure No. 6: Laminar air flow

Assay of extracts by disc diffusion technique:

The stock solution (each of 10µg/ml) of both extract and ciprofloxacin were prepared. From these stock solutions, two sets of 4 dilutions (100, 200, 400. 800 µg/ml) each extract (sterile distilled water) and ciprofloxacin (sterile distilled water) were prepared in sterilized McCartney bottles. Sterile Nutrient agar plates were prepared and incubated at 37^0C for 24h to check for the presence of any sort of contamination. Then each sterilized agar plates were flooded with liquid culture of bacterial strains, dried for 30 minutes at 37^0C. The sterile Whatman filter paper disc were soaked in four different dilution of the crude extract and placed in an appropriate position of the plates marked as quadrant at the back of Petri dishes. All the flooded plates with corresponding paper discs soaked with the appropriate dilution of extract were incubated at 37^0C for 24h and diameter of zone of inhibition and corresponding zone diameters will be measured and compared accordingly.

RESULTS AND DISCUSSION:

Extraction of plant material:

The plant material was extracted in Soxhlet apparatus and the percentage yield calculated by the following formula was found out to be 12.5%w/w.

$$\text{Percentage yield} = \frac{\text{wt. of extract- wt. of empty china dish} \times 100}{\text{wt. of powdered drug}}$$

PHYTOCHEMICAL INVESTIGATION

The results of phytochemical investigation are as follow:

Table No. 1: Result of Phytochemical Investigation

Phytoconstituents	Solvents			
	Methanol	Water	Petroleum ether	Chloroform
Tannin	++	-	-	+
Alkaloid	++	+	+	+
Carbohydrate	+	+	-	-
Proteins and amino acids	+	+	-	-
Saponin	+	+	-	-
Glycoside	+	+	+	-
Phytosterols	++	+	-	+
Phenol	++	+	+	+
Flavonoids	+	+	+	+
Diterpenes	+	+	+	+

Key: ++ means abundant; + Indicates presence; - indicates absence

It is evident from the results of phytochemical investigation that solvent methanol extracts maximum number of phytoconstituents.

Effect of methanolic extract of *Tinospora cordifolia* on castor oil-induced diarrhoea:

The effect of the extract was studied on control of diarrhoea by castor oil induced diarrhoea model.

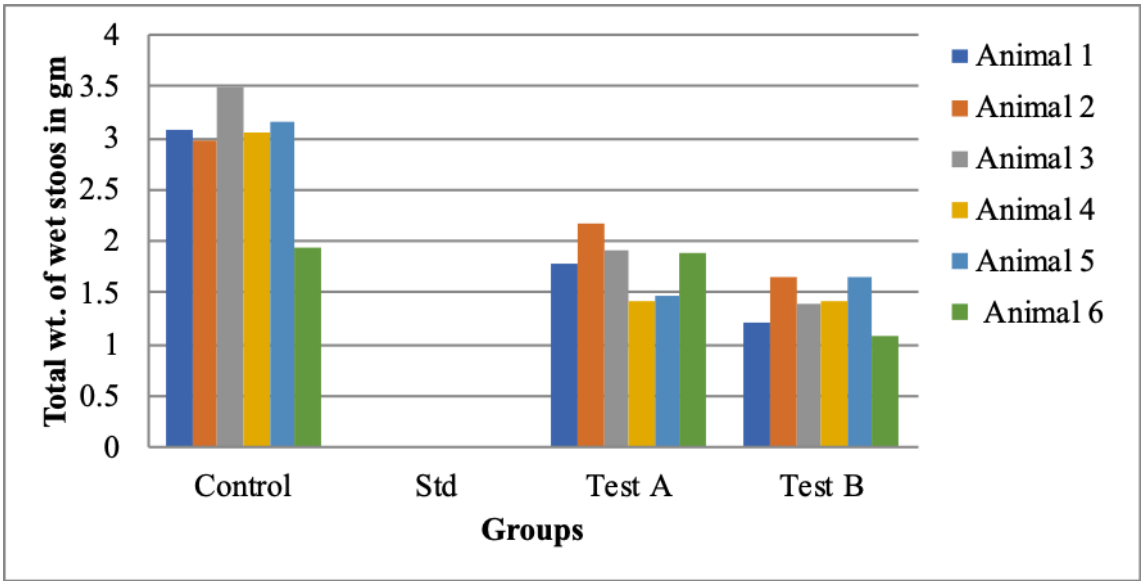
Table No. 2: Effect of methanolic extract of *Tinospora cordifolia* on castor oil induced diarrhoea:

Treatment group	Dose(mg/kg)	No of animals	Total weight of defecations (gm)	% inhibition
Control	-	6	2.95±0.15**	-----
Standard (Lopramide)	3	6	0	100
Methanolic extract (Test A)	200	6	1.77±0.01**	40
Methanolic extract (Test B)	400	6	1.22±0.18**	60

** = p < 0.01 = Very significant

Table No. 3: A table between total weight of wet stools and different groups.

No of animal	Groups			
	Control	Std.	Test A	Test B
Animal 1	3.092	0	1.793	1.202
Animal 2	2.965	0	2.18	1.659
Animal 3	3.503	0	1.919	1.403
Animal 4	3.057	0	1.416	1.015
Animal 5	3.158	0	1.458	1.001
Animal 6	1.946	0	1.88	1.074



Graph 1: A graph between total weight of wet stools and different groups.

The effect of the plant extract was studied on castor oil induced diarrhoea model. In these test healthy albino rats of either sex were divided into four groups of six animals each. They were fasted for 24h prior to the test, but allowed free access to water.

Group 1 Received control: Normal saline (0.9%w/v)

Group 2 Received standard drug (Lopramide 3mg/kg).

Group 3 Received dose of the extract (200mg/kg)

Group 4 Received dose of the extract (400mg/kg)

All doses were administered orally. The animals were then housed singly in cages lined with transparent paper. One hour after pre-treatment with the extract, the animals were challenged with 1 ml of castor oil orally. Stools were collected on nonwetting paper sheets of uniform weight up to 6 h after administration of the castor oil. Every 15 min during the first 8 h, urine was drained off by gravity, and the net stool weight, termed early diarrhoeal excretion, was recorded. The diarrhoea-free period is defined as the time in minutes between castor oil administration and the occurrence of the first diarrhoeal output. The acute diarrhoeal phase is the time between the first and the last diarrhoeal output of the 8-h observation period. Stools occurring between 8 and 24 h after castor oil administration are called late diarrhoeal excretion. Multiple comparisons of mean were carried out by one way analysis of variance (ANOVA), A probability level of less than 5% was considered significant.

The mean weight of wet stools of control group was found to be high as 2.954 while in standard group it was completely inhibited at a dose of 3mg/kg of standard drug Loperamide. In test group A and B the mean weight of wet stools was found to be 1.77 & 1.22 respectively. P value for all was found to be < 0.01. Thus it was observed that extract was having a highly significant antidiarrheal effect as evident from the P value.

From the experimental data obtained from Table 2, it was observed that the leaf extract at the dose of 200mg/kg and 400mg/kg showed significant antidiarrheal activity was found to be maximum at 400mg dose extract. It means that the effect was found to be vary in a dose dependent way.

***In-vitro* antimicrobial activity**

Antibacterial activity:

The result in Table 4 depicted the MIC values of the methanolic extract of *Tinospora cordifolia* against various tested bacterial pathogens.

Table No. 4: Determination of MIC of methanolic extract of *Tinospora cordifolia* against various bacterial strains

S. No	Name of microorganism	Dilution of methanolic whole plant extract (µg/mL) in nutrient agar media						
		0	5	25	50	100	200	400
1	*Pseudomonas auriginosa* AP585 NLF	+	+	+	+	+	+	+
2	*Pseudomonas putida* MTCC 2252	+	+	±	±	-	-	-
3	*Vibrio cholera* 426	+	+	+	+	±	±	-
4	*Vibrio cholera* 575	+	+	±	±	±	±	-
5	*Vibrio cholera* 765	+	+	+	+	+	+	+

6	*Vibrio cholera* 1023	+	+	+	+	+	+	+
7	*Vibrio cholera* 1033	+	+	+	+	±	±	-
8	*Vibrio cholera* 1311	+	+	+	+	+	+	+
9	*Vibrio cholera* BDI/81	+	+	+	+	+	+	+
10	*Shigella dysenteriae* 1	+	-	-	-	-	-	-
11	*Shigella soneii* NK 29	+	+	+	+	+	+	+
12	*Shigella soneii* BCH 397	+	+	±	±	±	_	-
13	*Shigella soneii* F11001	+	+	+	+	+	+	-
14	*Shigella boydii* 22461	+	+	+	+	+	+	+
15	*Shigella flexneri* type 36 NK 381	+	+	±	±	±	±	-
16	*Bacillus cereus* MTCC 1305	+	+	+	+	+	+	+
17	*Bacillus subtilis* MTCC 441	+	+	+	+	+	+	+
18	*Escherichia coli* 306	+	+	+	+	+	+	_
19	*Escherichia coli* 798	+	+	+	+	+	+	_
20	*Escherichia coli* 35B	+	+	+	+	+	+	_
21	*Escherichia coli* 18/9	+	+	+	+	+	+	_
22	*Actinobactor spp*	+	+	+	+	+	±	_

23	*Proteus vulgaris* AP769 NLF	+	+	+	+	+	+	+
24	*Klebsiella pneumoninae* 329	+	+	+	+	+	±	–
25	*Salmonella typhii* type II	+	+	+	+	+	+	+

0= control, ± Inhibited Growth, + Growth, - Absence of Growth

The analysis of Table 4 shows the antibacterial potentiality of the leaf extract. The extract was shown to inhibit three strains of *V. cholera* within a concentration of 100µg/mL. The pattern of drug resistance towards the extract is also revealed against various clinical microorganism treated in our study. *Pseudomonas auriginosa* AP585 NLF, some strains *Vibrio cholera*, *Shigella boydii* 22461, *Bacillus cereus* MTCC 1305 and *Salmonella typhii* type II were found to be completely resistant against the tested extract. The extract however showed potent inhibitory effect against *Shigella dysenteriae* 1 as the strain was inhibited at a concentration as low as 5µg/mL. It is observed from the study of Table 4 that the tested extract has prominent antibacterial effect against the gram-negative microbes especially those causing diarrhoeal disease in human especially *E. coli* and *Vibrio cholerae*. The extract was found to inhibit strongly *Klebsiella pneumonia* 329 and various strains of *E. coli* and *V. cholerae BDI/81* within 400 µg/mL and these strains on preliminary studies was found to show strong resistance against conventional antibiotics like ampicillin and ciprofloxacin. Thus it is a point to be noted that the extract has the potentiality to inhibit various multidrug-resistant hospital cultures isolated from patient.

The strains for disc diffusion study were selected on the basis of their results of MIC. The sensitive strains were tested for the disc diffusion analysis as shown in Table 5 and Graph 2.

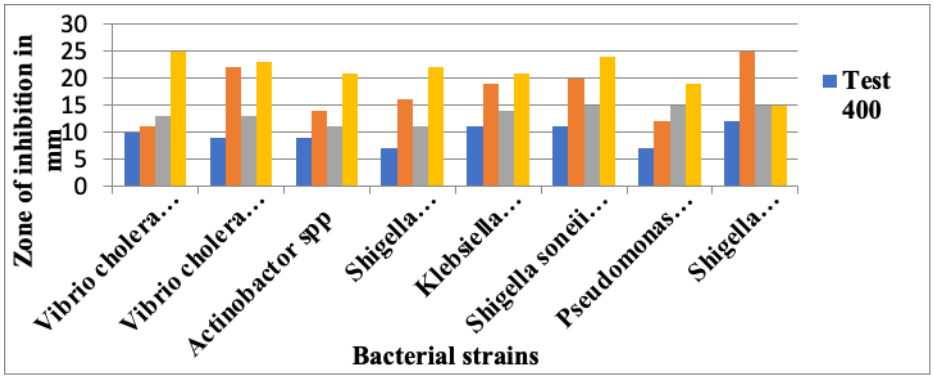
The result of determination of zone of inhibition of the leaf of *Tinospora cordifilia* and its comparison with standard antibacterial agent ciprofloxacin against the bacterial strains is recorded in the above Table 5.

The result of determination on zone of inhibition of crude extract and comparison with standard antibacterial agent ciprofloxacin against the bacterial strains is recorded in the Table 5.

Table No. 5: Determination of diameter of zone of inhibition (mm) produced by the methanolic extract of *Tinospora cordifolia* and its comparison with Ciprofloxacin against selected sensitive bacterial strains

S. No.	Name of bacteria	Zone of inhibition (in mm) against various doses of extract and standard drug			
		Extract (µg/mL)		Ciprofloxacin (µg/mL)	
		400	800	400	800
1	*Vibrio cholera* 575	10	13	11	25
2	*Vibrio cholera* 1033	9	13	22	23
3	*Actinobactor spp*	9	11	14	21
4	*Shigella flexneri* type 36 NK 381	7	11	16	22
5	*Klebsiella pneumoninae* 329	11	14	19	21
6	*Shigella soneii* BCH 397	11	15	20	24
7	*Pseudomonas putida* MTCC 2252	7	15	12	19
8	*Shigella dysenteriae* 1	12	15	25	26

(* All the values are in mm)



Graph 2: A chart showing the zone of inhibition of various concentration of extract of *Tinospora cordifolia* against test organisms.

Shigella dysenteriae 1 and *Shigella soneii* were found to be most sensitive strain amongst all *Shigella* tested spp as that were found to be strongly inhibited by the extract even at a concentration of 5µg /mL and 25µg/mL. Only two strains out of 6 tested strains of *Shigella spp* showed drug resistance plasmid as it was found to grow even in presence of 400µg/mL of the methanol extract of *Tinospora cordifolia*.

Vibrio spp. was moderately inhibited. *Vibrio cholera* 575, *Vibrio cholera* 1033 and *Vibrio cholera* 426 and *Vibrio cholera* BDI/81 showed no growth at 400 µg/mL concentration of the extract. *Vibrio cholera*, 765 *Vibrio cholera* 1023 and *Vibrio cholera* 1311 were found to be completely resistant against tested extract at the higher tested concentration. All strains of *E.coli* were found to be inhibited at 400µg/mL concentration of the extract.

The tested *Actinobacter spp* and *Klebsiella pneumoninae* 329 showed inhibition at 200µg/mL. However strain of *Salmonella typhi* II and *Proteus vulgaris* were found to be unaffected by the tested extract. Thus the *in vitro* antimicrobial activity of methanolic leaf extract of *Tinospora cordifolia* was studied against Gram-negative (*E.coli, Shigella spp, Vibrio spp*) and Gram-positive (*Bacillus spp, S. aureus*) organisms. On comparing the activity with that of a standard antibiotic (Ciprofloxacin) it was found that it showed an excellent inhibitory effect on the growth of Gram negative microbes predominantly.

DISCUSSION:

Tinospora cordifolia commonly known as Giloy or Guduchi is a well-known plant used in traditional system of India medicine. This is widely used in the treatment of dysentery, leprosy, jaundice, cutaneous rashes and impotency. Therefore to justify the traditional uses of plant we have scientifically screened the whole plant of Tinospora cordifolia for its antidiarrhoeal and antimicrobial potentiality.

The methanolic extract of whole plant of *Tinospora cordifolia* reveals the presence of various phytoconstituents including glycoside, saponin, alkaloid, tannin, proteins and amino acids. The extract was found to very active against various gram negative strains of microorganism including *Vibrio cholera, E. coli, Shigella dysenteriae* 1 and *Klebsiella pneumoninae*. Extract was also found to show action against that microorganism which causes diarrhoeal disease in human beings. Majority of the strains showed action within a concentration of 400µg/mL. The extract was found to be very active against those strains whose were resistant to conventional antibiotics like penicillium and ciprofloxacin. Thus it is a point to be noted that

the extract has potentiality to inhibit various multidrug hospital culture isolated from patient. The extract was found to inhibit the microorganism in a dose dependent manner and the activity was comparable with a dose of ciprofloxacin. The extract was found to be highly active against various strain of *Candida albicans* tested. All the selected fungal strain was inhibited within a concentration 400µg/mL with the exception of *Aspergillus fumigatus* which was inhibited within 400µg/mL in SDA media. The strength of extract was comparable with that of griseofulvin when tested in vitro. Therefore the result suggested that the whole plant of *Tinospora cordifolia* exhibited potent antidiarrheal and antimicrobial activity.

CONCLUSION:

Tinospora cordifolia widely used in the treatment of dysentery, diarrhoea, leprosy, microbial, jaundice, cutaneous rashes and impotency. Therefore to justify the traditional uses of plant we have scientifically screened the leaf of *Tinospora cordifolia* for its antidiarrheal and antimicrobial potentiality. The methanolic leaf extract of *Tinospora cordifolia* reveals the presence of various phytoconstituents including glycoside, saponin, alkaloid, tannin, flavonoids, phytosterol, diterpenes, proteins and amino acids. The extract was found to very active against various gram negative strains of microorganism including *Vibrio cholera, E. coli, Shigella dysenteriae 1* and *Klebsiella pneumoninae*. Extract was also found to show action against that microorganism which causes diarrhoeal disease in human beings. Majority of the strains showed action within a concentration of 400µg/mL. The extract was found to be very active against those strains that were resistant to conventional antibiotics like penicillium and ciprofloxacin. The extract was found to inhibit the microorganism in a dose dependent manner and the activity was comparable with a dose of ciprofloxacin. The extract was found to be highly active against various strain of *Candida albicans* tested. Therefore the result suggested that the whole plant of *Tinospora cordifolia* exhibited potent antimicrobial activity. The extract was also found to show potent antidiarrheal activity at a dose of 200 and 400mg/kg body weight. The activity was compared with the standard drug loperamide.

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